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GUM-PRODUCING ORGANISMS IN SUGAR CANE

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The importance of the gummosis disease of sugar cane and the advance in our knowledge of the subject since the publication of the previous papers by the senior author have made a further study of the subject very desirable. The most important of these advancements is in our knowledge of the geographical distribution and of new species or strains.

HISTORY

This disease was reported first from Brazil by Dränert in 1869 but appears to have been known as early as 1863. It was reported next from Australia by Cobb who described the organism in 1894. He believed it to have been in the country as early as 1876. The wide separation of these two countries presented a very interesting problem in geographical distribution. Shepherd, of Mauritius, in his report for the International Survey of the Diseases of Sugar Cane (1932), said that it was probably introduced from Brazil to Mauritius on a shipment of seed cuttings in 1869 and from there to Australia in 1874. North, of Australia, in his report for the same survey, says that it was probably introduced into Australia from Mauritius in 1874. The next report was from Brazil in 1894, but it was less severe than in 1869, probably due to the use of resistant varieties. It was found in Puerto Rico by Matz in 1920 and reported the same year. It was discovered in St. Kitts of the British West Indies in 1925 and reported by Ballou in 1926. It was found and reported from Colombia by Chardón of Puerto Rico in 1926. It was found and reported in Guadeloupe of the French West Indies by Williams

[1] This is the third paper on the gummosis of sugar cane by the senior author. Early in 1932 the junior author began to cooperate in the work and has made the studies on the reaction of sugars to these organisms and also the serological studies.

(**16**) in 1929. It was reported from Dominica of the British West Indies by Ashby (**1**) in 1928–29; from St. Lucia of the British West Indies in 1929, and from Antigua of the British West Indies by Illingworth in 1930.

In addition to the above places it has been reported from Fiji Islands, Java, Borneo, Reunion and New Guinea. The report from Java by Groenewege in 1915 was proven to be a mistake by Wilbrink who published the results of her studies in 1920. The disease which was supposed to be gummosis was found to be what is now known as scald (*Phytomomas albilineans* Ashby). The reports from Borneo and New Guinea have not been confirmed.

The symptoms of the disease have been described by many students of the subject. The descriptions by the various authors are very harmonious. The senior author in his description which was published in 1928 makes the following statement:

> "The most reliable external symptom is the leaf streaks referred to by Matz and Cottrell-Dormer. However, this symptom is sometimes present on POJ canes, and occasionally on Uba and some other canes, although it is impossible to find any trace of gumming in the cut surfaces."

Again in a paper read before the plant-pathology section of the Fourth Congress of the International Society of Sugar-Cane Technologists (1932) (**8**) he said,

> "Although the presence of these stripes is the most important external symptom I am satisfied that it is not always reliable. I have found infected canes which did not show these symptoms and I have found these symptoms in canes which did not show internal gumming or discolorations."

Further studies in Puerto Rico on the symptoms of this disease following the publication of this first paper led to more confusion. The leaf symptoms appeared in several resistant varieties which did not show discolorations or gum in the fibro-vascular bundles of the stems or bacteria in the leaf stripes. Finally the senior author began referring to cases of this kind as "false gummosis". This phase of the problem was solved at the Fourth Congress of the International Society of Sugar-Cane Technologists which met in Puerto Rico, March 1932, when it was found that this "false gummosis" was the same as a disease reported by Dr. Wilbrink of Java in 1929 as "fourth disease" and by Martin of Hawaii in 1930 as "chlorotic streak." It was also learned that this disease had been noted by Bell of Australia who referred to it as "false scald".

The leaf symptoms of this fourth disease or chlorotic streak are very similar to the leaf symptoms of the gummosis. The streaks

may be somewhat broader than in gummosis and the boundaries may be somewhat wavy. As a rule the reddish dots which are characteristic of the gummosis do not develop in fourth disease or chlorotic streak, but there is a reddish margin on the older streaks.

The exudation of gum on the cut surfaces of the canes is the most reliable symptom of the disease. The diseased fibro-vascular bundles are usually discolored, the most common discoloration being red but a discoloration does not necessarily indicate the disease and the absence of gum on the cut surfaces does not necessarily indicate that the cane is free from the disease. During periods of very dry weather diseased canes may show discolorations and but little or no gum. During or following periods of wet weather the canes will show the gum in varying amounts, depending on several factors, such as weather conditions, length of time that the cane has been infected and variety of cane.

Within the past few years certain variations in the morphologic and physiologic characters of the organism have been reported which make further study desirable.

The writer sent cultures to Mr. M. C. Goldworthy of the University of California in 1927 who compared them with cultures sent from Australia by Mr. A. F. Bell for that purpose. Under date of June 27, 1927, he replied as follows:

> "Your vascular types are different from those we have received from Australia. That is they behave differently on media. So far I have had no opportunity of comparing the cultures by the serological method."

Ashby (**2**) (1929) published the results of a study of this disease in the British West Indies in which he reported the finding of two organisms. He said:

> "Plantings in peptone saccharose agar made with yellow ooze from the stalks of affected canes in St. Kitts yielded two types of colonies, these, the more rapidly appearing (3 to 4 days) being entire convex, glistening and, at first, colourless but later pale yellow and spreading: the later appearing colonies were entire, flat, deeper yellow, and the growth more restricted. The first type of colony yielded a straw or amber yellow (Ridgway) abundant slimy growth on slanted agar in tubes with a marked tendency to run down and accumulate at the bottom of the slope; after inoculating into milk a shallow clear zone formed at the top of the liquid in two or three days at tropical room temperature with a bulky indistinct clot apparently due to a labenzyme and the reaction became increasingly alkaline. The second more slowly growing type of colony which was yellow from the start yielded a restricted glistening aniline to primuline yellow growth on the agar with a compact slime showing little tendency to flow. In litmus and plain milk no change occurred in a week but there was gradual increase of alkalinity subsequently. As the organism in the first type of colony

showed cultural characters different from those of *B. vascularum* as described by Erwin F. Smith and as those of the second type were in agreement with his description the first type was discarded and attention given to the second."

He secured cultures from North of Australia and made inoculations in cane in England which enabled him to compare the St. Kitts organism (second type) with Australian organism. The Australian type produced broader stripes, a withering of the heart and a rotting of the apical internodes. It was more severe than the St. Kitts second type. He said:

"The original cultures of the St. Kitts organism (second type of colony) gave rise to a glistening restricted growth on peptone-saccharose agar (saccharose 2.0, with peptone 0.5, dipotassium phosphate 0.05, magnesium sulphate 0.025, agar (bacto) 1.5, water 100.) between aniline and primuline yellow in colour (Ridgway); the slime was compact drawing out, in cultures which had attained their full growth, into elastic threads and showing little tendency to flow. The growth was opalescent in oblique light and gelatine (10 per cent.) of the same composition as the agar medium was slowly liquefied after two weeks. Lavender-colour litmus milk became gradually strongly alkaline and after two to three weeks at 23° C. began to clear from the surface with bleaching. After two to three months the milk had cleared with more or less suspended slime and restoration of the litmus colour but remained permanently strongly alkaline. If the milk carried a layer of fat a yellow growth developed on the surface and there was little deposit; in the semi-anaerobic conditions under the layer of fat, bleaching of the litmus was complete for a time, and it appeared that action on the milk was mainly by diffusion of metabolic products through the fat layer. In well-separated milk, surface growth was restricted to a yellow ring, there was active multiplication within the liquid and a good yellow deposit associated with partial bleaching and more rapid restoration of the litmus colour. It is doubtful if, at any time, a true clot was formed, the eventual clearing being apparently due to increasing alkalinity since cleared cultures yielded a bulky precipitate when acidified with hydrochloric acid. The organism which was actively motile in young liquid cultures by means of a single polar flagellum exceeded 0.5 microns in diameter and was from 1.0 to 1.5 microns in length occurring singly, in pairs and more rarely in short chains. Most of the isolations showed colonies similar to those of the original isolation but those from the leaf stripes of Uba were dry, flat, rough and pale yellow with wavy margins, the growth from these colonies on agar slants was at first similar but tended gradually to take on the glistening smooth deeper yellow character of the original form. Some of the cultures from the isolations caused, like the original, a slow liquefaction of gelatine but others showed no trace of liquefaction at room temperatures after three months. The action on milk varied in the rate of change but there was no true clot and all eventually cleared with persistent alkalinity.

"The original culture from Australia gave rise on the agar to convex glistening almost colourless colonies of a fluid slimy consistency which became paler yellow and spreading. On agar slants the growth was abundant, opalescent and fluid slimy with a tint from straw to amber yellow: the slime tended to flow and accumulate at the base of the slant. On potato the growth was sulphur yellow

fluid and flowing off the surface but not so abundantly as the slime of *B. Malvacearum* E. F. Smith. Gelatine showed liquefaction under a week and a shallow clear zone formed at the surface of plain and litmus milk after three to four days at 23° C associated with the formation of a bulky indistinct clot apparently due to a lab-enzyme; alkalinity gradually increased becoming strong and persistent and the milk was completely cleared after two to three months of 23° C; the clearing appeared to be caused by peptonisation as no precipitation followed acidification with hydrochloric acid. Growth occurred as a yellow slimy layer on the surface of milk with fat present and absent. The organism was actively motile in young cultures and approximated in size to that of the St. Kitts form.

"The colonies which appeared in platings made from the inoculated canes were not uniform in type; some resembled those of the original culture, but they were as a rule, mingled with others showing a piled-up deeper yellow colour, a dark opaque nucleus, and little tendency to spread; others again were at first flat, but changed to the second type after a few days. Transfers from the different colonies to agar slants yielded, however, a similar pale yellow slimy fluid growth like that of the original form. All cultures from isolations behaved like the original culture in liquefying gelatine appreciably within a week and clotting milk in three to four days at 23° C. with gradually increasing alkalinity and in clearing it eventually but with marked differences in the rate of clearing as cultures from the second or 'piled' up type of colony were slower in action. The isolation from Australia, showed therefore, cultural characters similar to those of the first type from 'gummed' cane in St. Kitts, and it is believed now that they are probably identical. As the cultures made from the isolations out of the different varieties were essentially similar to the two types used for inoculation, and as no evidence was obtained that the one type could change into the other, it would appear that two yellow forms may cause Gummy Disease occurring in some instances together and which differ in cultural characters enough to be considered as distinct varieties of *B. vascularum*. The strain described by Erwin F. Smith resembles the second type from St. Kitts differing from some cultures of it only in not eventually clearing milk, a difference which appears to depend on the amount of alkali produced; the alkaline body is either ammonia or an amine as the vapour from boiling cultures turns red litmus paper blue."

In August, 1930, a gummosis disease was discovered on POJ 2878 at Jayuya, a point located in the center of the island and at an elevation of about 2,000 feet. This was especially interesting because this variety was supposed to be immune or highly resistant.

The cane was sent to the laboratory and put in a moist chamber, where it produced a typical gumming within 24 hours. Owing to the fact that this variety was supposed to be immune, the senior author and Mr. Pedro Richardson, Agronomist, visited Jayuya in order to make sure that it was POJ 2878. After a careful examination, it was decided that there was no doubt as to the variety. The symptoms were not quite typical. However, the gumming was so pronounced that it was detected by the foreman who was making cuttings for planting. The behavior of the organism in culture was typical of *B. vascularum*.

This outbreak was described by the senior authority---------- as follows:

"The cane was about seven months of age from date of planting and the infection was well over 75 per cent.

"The external symptoms were somewhat different from those described by the various writers on the subject. The early symptoms were not found but the late symptoms were very abundant. These symptoms consisted in a dying of the tissues in the leaves along the veins, thus producing elongated areas of dead tissue which frequently extended to the leaf margins. In many cases the margins of the leaves also were dead. When the tops of the canes were cut across, many of them showed a gumming, which was much more pronounced when the cuttings were kept in moist chamber for a few hours.

"The gum was not the typical honey yellow which has been described by several writers, but ranged from clear to creamy white or yellow, and in some cases was slightly tinted with honey yellow."

The further history of this outbreak is as follows:

A field test with healthy P.O.J. 2878, 2883, 2714, 2727, P.R. 801, 803, 807, 809, 820, 826, F.C. 916 and Guadeloupe 119 was started in which every third row was planted with infected P.O.J. 2878, so that every variety of healthy cane was in contact with an infected row. The rows were five feet apart and consisted of 50 stools each. The field of infected cane was plowed out.

At this same time cuttings of the same varieties were set in our greenhouse, and the young canes inoculated with cultures prepared in the laboratory. Some of P.O.J. 2878, 2883, P.R. 807, 809, 826, F.C. 916 and Guadeloupe 119 developed slight symptoms and the organism was recovered from them but in an attenuated form. After a time the new growths failed to produce symptoms and it was impossible to recover the organism.

The field test was cut January 20th, 1932, and carefully examined by the writer. Neither symptoms nor gumming were found in any of the varieties, nor in the rows planted with infected cane. A few canes showed slight discolorations of the fibro-vascular bundles, but no gumming. They were brought to the laboratories and used for cultures. P.O.J. 2883 and F.C. 916 produced a gum organism which is not typical. The color of the gum is greyish, almost clear; some times tinted with yellow.

The greenhouse tests made by the writer with organism from P.O.J–2878 at Jayuya lead the writer to believe that P.O.J.–2878, P.O.J.–2883, P.R.–807, P.R.–809, P.R.–826, F.C.–916 and Guadeloupe 119 may be symptomless carriers although this has not been demonstrated. However, the studies during 1933 show that P.O.J.–2878 when inoculated with an extremely virulent strain of *B. vas-*

cularum will sometime develop leaf symptoms. It is the opinion of the writer that these varieties are so resistant as to be practically immune and that the experiments indicate that it is possible for a very highly resistant or apparently immune variety to be a carrier of this disease.

Cultures for P.O.J. 2878 were sent from Puerto Rico by the writer to Mr. A. F. Bell of Australia for comparison with the Australian organism. He replied as follows:

"I desire to acknowledge receipt of your letter of 22nd October, also of four cultures. Of the latter three were apparently pure and one contained a yeast. The former were re-isolated and examined and compared with the organism of leaf-scaled, gumming, red stripe, and mottle stripe. Your organism is quite distinct from any of these four.

"Parallel inoculations were made (in the transfer chamber) into cut shoots of Badila and from this small test your organism seemed much more virulent than the above four stains.

"At the end of two days the lesions were about one-fourth inch in diameter and consisted in dark-red rings surrounding a water-soaked greenish or yellowish area. Later the centers became ashy coloured and the red ring surrounded by a yellowish halo.

"I enclose a photograph of the lesions. The cultures and experimental material have now been destroyed. Our experience so far it that P.O.J. 2878 is highly resistant to gumming disease."

The senior author wrote another letter to Mr. Bell making inquiry as to the morphological characters of the Puerto Rican and Australian organisms. He replied (under date of March 30th, 1931) as follows:

"Your letter of 10th February to hand. With reference to the organism you sent, this was quite distinct from our *B. vascularum* both culturally and morphologically. One considerable difference was that the organism received from you had flagella at both poles while the gumming organism has a singled flagellum only. The organism to which it bore most resemblance was that causing mottle stripe."

An examination of the photograph (Fig. 2) of this organism, made by Mr. Bell, shows that it is quite distinct from *B. vascularum*. The studies by Ashby and by the senior author emphasize the importance of more extensive studies on this disease and its cause or causes in different parts of the world.

1932 Studies

There was a severe outbreak of gummosis on the small island of Vieques in March and April 1932 and slight outbreaks in the vicin-

ity of Río Piedras and Canóvanas. The senior author made a special study of infected Cristalina from the island of Vieques on a very small mixed planting near Canóvanas and on mixed plantings containing Cristalina near Río Piedras during 1932. Vieques is a small island just east of Puerto Rico and has much less rainfall than Puerto Rico. Cristalina has been retained there as the variety of major commercial importance long after it gave way to other varieties in Puerto Rico. It is very susceptible to this disease and the infection is very near to 100 per cent. The rainfall was exceptionally high during the spring of 1932 and the disease was very evident. Most of the cuttings showed a high yield of gum.

Many cultures were made and studied. The results confirmed the opinions of the senior author which were published in 1928. There were many strains which varied in color and character of growth. These results are shown in table I.

TABLE I.

No.	Source	Color	Growth on new cul.	Inoc. 6/20 Symptoms 6/24	June 18th Symptoms	July 1 Symptoms	July 18 Symptoms
1	Australia.....	Yellow ...	Poor.....	POJ-2878 none	Crist none..	POJ-2878 none	None
2	Jayuya FC-916.....	White	Poor.....	POJ-2878 none	Crist none..	POJ-2878 none	None
3	Vieques 6032.....	Canary Yellow	Poor.....	POJ-2878 none	Crist none..	POJ-2878 none	None
4	Vieques Crist.....	Yellow ...	Good	POJ-2878 none	Crist none..	POJ-2878 none ...	None
5	Vieques.....	Yellow ...	Fair	Very Slight	Crist none..	POJ-2878 none	None
6	Vieques.....	Yellow ...	Good	Good	Good	POJ-2878 none	Slight
7	Vieques.....	Yellow ...	Good	Very Slight.	Very Slight.	Slight	None
8	R. P. Crist.....	Yellow ...	Good	Good	Good.....	18 in.	Slight
9	Jayuya POJ-2878..	White	Poor	Very Slight.	Slight	1¼ in.....	Slight
10	Vieques.....	White	Good	Slight	Very Slight.	Outgrown	None
11	Vieques.....	Yellow ...	Fair	Slight	None	Outgrown	None
12	R. P. H.—109 slow..	Yellow ...	Fair	Slight	Very Slight.	Outgrown	None
13	R. P. H.—109 slow..	White	Poor	None.....	Very Slight.	Outgrown	None
14	R. P. H.—109 slow..	White	Poor.....	Slight	None	None.....	None
15	R. P. H.—109 slow..	White	Good	Slight	Very Slight.	Outgrown	None

R. P. H.—109 = H 109 from Río Piedras.

The results given in this table confirm the opinion of the senior author expressed in 1928 that this species included a large number of strains which varied in color, growth and virulence.

1933 Studies

The first half of 1933 on Vieques was very dry as compared with 1932. The Cristalina and Rayada (a variety of Cristalina) canes were very heavily infected with *B. vascularum* but the exudation of gum was much less than in 1932. A large number of isolations were made and used for laboratory study. All the cultures used in 1933 were new and from Vieques canes except three: Nos. 1 and 2 were 1932 cultures which had lost their virulence but made excellent growths on agar. No. 23 was a culture sent to the senior author by Mr. A. F. Bell of Brisbane, Australia, late in 1932 and received in January, 1933.

The laboratory studies showed a large number of strains which possessed the following characters:

1. *Color.*—Various shades of yellow and milk white, while others were clear or sometimes clear and slightly tinted with yellow. A few were brownish. In many cases the first growth of a yellowish exudation was white when transferred to agar. Many strains changed color on the agar. The strains were grown on different media and at different pH but up to the present time the changing of colors has not been explained. The best growths were made in acid media but good growths were made on alkaline media as high as pH 9.6 although it was slower than on the acid media. Strains also changed from rough to smooth and smooth to rough without any apparent cause.

The variations in color were in harmony with those reported by the senior author in 1928. Cobb reported variations in color in 1905 but other writers did not give much attention to this phase of the subject. Possibly the material which they were studying did not show the extensive variations which are reported by the authors of this paper.

Some strains were extremely virulent while others were slightly virulent as shown by the leaf symptoms. Others did not produce leaf symptoms but grew in the canes as was shown by the exudations when the canes were cut and kept in a warm, moist chamber.

There was very little relationship between color and virulence although in general it may be said that the yellow strains were slightly more virulent than the others.

No experiments were made to determine temperature relationships but cultures that were put in incubators and refrigerators died in a short time.

Two series of inoculations were made and recorded in Table II. Other inoculations were made but the results were the same as shown in this table.

TABLE II.

No.	Color	
1	Yellow	No infection. Was virulent in 1932.
2	White	No infection. Was virulent in 1932.
3	White	No infection.
4	Almost clear	No infection.
5	White, yellow with age	Mild symptoms on Cristalina.
6	Yellow	Symptoms on Cristalina and H-109.
6	White	Symptoms on Cristalina and H-109.
7	Yellow	Symptoms on Cristalina and H-109 and POJ-2878.
8	White, yellow with age	Not infectious.
9	Clear, brown with age.	Not infectious.
10	White, brown with age	Not infectious.
11	White	Mild symptoms on Cristalina.
12	Whitish, almost clear.	Not infectious.
13	Yellow	Symptoms on Cristalina.
13	White	Symptoms on Cristalina.
14	White	Not infectious.
14	Yellow	Not infectious.
15	White	Very slight symptoms on Cristalina. Developing very slowly.
16	Clear, yellow tint.	Mild symptoms on Cristalina and H-109.
17	Clear, yellow tint.	Mild symptoms on Cristalina and H-109. Developing very slowly.
17	Yellow	Not infectious.
18	White	Not infectious.
19	White	Symptoms on Cristalina and H-109.
22	White	Symptoms on Cristalina and H-109 and POJ-2878.
23	Yellow	Very mild symptoms on Cristalina.
24	Clear, white tint.	Symptoms on Cristalina, H-109 and POJ-2878.
25	White	Symptoms on Cristalina and PR-803.
26	Yellow	Symptoms on Cristalina.
27	Yellow	Symptoms on Cristalina, H-109 and POJ-2878. Very slight symptoms on FC-916.
28	Light yellow	Symptoms on Cristalina, H-109 and very slight symptoms on M-28.
29	White	Symptoms on Cristalina.
30	Yellow (rough)	Symptoms on Cristalina and POJ-2878.
31	Yellow (smooth)	Symptoms on Cristalina. Slight symtoms on PR-803.
32	White	Symptoms on H-109.
32	Clear	Symptoms on Cristalina.
33	White	Symptoms on Cristalina.
34	Yellow	Symptoms on Cristalina.
35	Yellow	Slight symptoms on Cristalina.
36	Yellow	Symptoms on Cristalina.

Nos. 1 to 23 inoculated 5/5/33. Nos. 24, 36, inoculated 5/19/33. Time for appearance of leaf symptoms one to three weeks, occasionally longer. Nos. 1 and 2 were 1932 culture which lost their virulence. No. 23 from Australia. All others were 1933 cultures.

METHODS OF INOCULATIONS

Several methods of inoculation were used as follows:

1. Pricking the cultures into young cane or leaves with a needle. This was successful but slow and the percentage of takes less than with the other methods.

2. Cutting of the tops of the canes almost down to the growing points and the immediate application of the organism in agar. Then covering the mass with a pad of wet cotton. These inoculations were made late in the afternoon so that the agar would remain moist as long as possible. In some cases distilled water was poured on the cotton one or more times during the following day. The inner leaves push upward within 48 hours and if the variety is very susceptible white streaks will be found running downward. Varieties that are practically immune will not show these streaks. Sometimes streaks

three or four inches in length, will develop in highly resistant variety. However, they do not lengthen after the first few days and the organism dies.

3. Cutting a small hole into the spindle above the growing point and the insertion of the organism from an agar culture. Within ten days or two weeks the injured parts of the leaves pushed out and unrolled. The presence of the organism could be determined by the development of white streaks in the leaves running up and down from the point of injury. The variations in time depended on the resistance of the variety and the virulence of the strain in the culture.

4. The inoculation of the organism from agar cultures into the stem below the growing point. This method was successful but slow. A high percentage of gum pockets resulted.

5. Removing the upper half of an advanced cane so as to force the development of side shoots. When the side shoots are well advanced cut holes in the old cane and insert agar containing the organism.

6. The insertion of pieces of diseased cane into slits in the spindle or cane. The results were practically the same as when agar cultures were used but more uncertain.

7. Inoculation of seed cuttings by the insertion of the organism from cultures in holes cut in the seed pieces. This method was slow and the results irregular.

The results of the inoculations not given in the Table II may be summarized as follows:

Rapid-growing canes respond to inoculations much more readily than slow-growing canes.

Canes inoculated with a culture may show negative results at one time and mild symptoms at another time. The symptoms may develop more slowly in some cases than in others although the inoculations are made from a single culture and on one variety. Some strains do not produce leaf symptoms but do produce gum in the canes and in cultures.

When an infected cane is cut across and placed in a warm, moist chamber, the gum oozes out on the cut surfaces, sometimes in such great abundance as to cover the entire cut area. Two or more colors may emerge from a single piece. When the gum is transferred to agar plates, it makes a very rapid growth. Sometimes all the colors persist and sometimes the deep yellow makes a clear growth. Some of the clear growths from yellow gum become yellow later and some remain yellow. The yellow strains show a great tendency to produce modifications of yellow and sometimes become clear or white. Some

strains grow much more luxuriantly than others. Some strains are much more virulent than others. Some strains that produce gum in the cane and in culture do not produce leaf symptoms. Positive results may be obtained from cultures of all colors and all tints but in general the yellows are more virulent than the other colors.

Bacteriological Study

Twenty-seven cultures collected in 1932 and previous to that date were studied and classified as follows:

Group I.—Is composed of five cultures isolated from the island of Vieques. They produced an abundant canary-yellow growth in twenty-four hours. They are bacilli arranged parallel and side to side, motile and show no spores nor capsules. The colonies are homogeneous, entire edged, straw in color, stain negative to Gram, growth in broth is turbid, agar stroke is slow, confluent, smooth, viscid and opaque and show no change in litmus milk. It does not ferment dextrose, lactose, dulcite, manite, maltose, sucrose, xylose or arabinose; does not produce hydrogen sulphide nor indol; the V.P. and M.R. reactions are negative; does not reduce nitrates, has no odor and emulsify very poorly. This group of organisms is identical with the cultures of *B. vascularum* isolated in Australia that we had previously studied.

Group II.—This group consisted of twelve cultures isolated from "Cristalina" cane in Vieques and from "F.C.-916" cane in Jayuya. Some produced a whitish-gray growth while others had a lemon-yellow growth. They are bacilli arranged side by side, parallel or end to end, motile, have no spores nor capsules. The colonies are homogeneous, entire edged, and straw in color. They are negative to Gram stain, grow slowly in broth with slight turbidity. In agar the growth is slow, confluent, raised, smooth, viscid and opaque. There is no change in litmus milk, no acid is produced in arabinose, xylose, dextrose, lactose, dulcite, manite, maltose and sucrose. They produce no hydrogen sulphide and no indol; the V.P. and M.R. reactions are negative; they do not reduce nitrates, do not produce any odor and have a poor emulsificability.

Group III.—Is composed of ten cultures isolated from "P.O.J.-2878" at Jayuya and "Cristalina" cane from Vieques. The growth of some of the cultures is absolutely colorless and in others is enamel white. They are bacilli which are found singly or in short chains, motile, form no spores nor capsules and are negative to Gram stain. The colonies are granular, straw in color and have entire edge. The

growth in broth is slight turbid with filament and at times a ring adherent to the tube is formed. The agar stroke is filiform, elevated, smooth and translucent or white. They do not ferment dextrose, maltose, manite, xylose, sucrose, arabinose or dulcitol; they do not produce hydrogen sulphide and the V.P. and M.R. reactions are negative. They do not reduce nitrates, have no odor and have poor emulsificability.

The cultures studied differ in pigment production but their sugar fermentations are similar. In 1933, eighteen cultures have been studied, ranging in color from yellow, white, creamy, lemon-yellow, grayish white to colorless. Thirteen of the cultures are bacteriologically similar to the description given in Group I of the 1932 cultures, that is, they are Gram negative organisms, motile, do not ferment any of the sugars tested, do not produce hydrogen sulphide, have no change in litmus milk, do not produce indol, do not reduce nitrates, the V.P. and M.R. reactions are negative, they have no odor and have very poor emulsificability.

TABLE III.

FERMENTATION REACTION, 1933 CULTURER

Culture B	Isolated from	Color	Glucose	Manite	Lactose	Xylose	Saccharose	Maltose	Dulcite	Arabinoses
23	Australian culture	Yellow								
24	Cristalina	White								
25	Cristalina	White								
26	Cristalina	White	+	+		+	+	+	+	+
27	Cristalina	Yellow								
28	Cristalina	Creamy yellow								
29	Cristalina	White								
30	Cristalina	Creamy yellow								
31	Cristalina	Creamy yellow								
32	Cristalina	Colorless								
32 c	Cristalina	Yellow								
33	Cristalina	White	+	+		+	+	+	+	+
34	Cristalina	Yellow								
35	Cristalina	Grayish white								
36	Cristalina	Grayish white								
37	Cristalina	White	+	+		+	+	+	+	+
38	Cristalina	Yellow	+	+		+	+	+	+	+
39	Cristalina	White	+	+		+	+	+	+	+

There are five cultures labeled 26, 33, 37, 38 and 39 which range from white to yellow color and which are absolutely different from the others macroscopically. They are bacilli arranged singly, motile, form no spores nor capsules. The colonies are finely granular, straw in color and entire edged. They are Gram negative. The growth in broth is heavy and granular; agar stroke is filiform, elevated, smooth and whitish in color. They produce slight acidity and coagulation in milk. They produce acid and gas in glucose, manite, xylose,

sucrose, maltose, dulcite and arabinose. They do not ferment lactose. They produce hydrogen sulphide; indol is negative; V.P. and M.R. reactions are negative; they have no odor and emulsify very poorly. These cultures produce a gummy substance but it is still questionable to our mind if they produce true gummosis.

Serological

Two rabbits were inoculated intravenously with cultures 32 c and 33, respectively, and two potent antisera were obtained. By direct agglutination cultures 23, 24, 25, 27, 28, 29, 30, 31, 32 and 32 c, 34, 35 and 36 agglutinated to different titers with antiserum 32 c, while they did not agglutinate at all with antiserum 33. The same was true of cultures, 33, 26, 37, and 39, which agglutinated with antiserum 33, but did not agglutinate at all with antiserum 32 c. This demonstrates that 13 cultures are antigenically alike, one of these strains, 23, being a known gummosis strain isolated from Australia. The other five cultures, i.e., 33, 26, 37, 38 and 39, are antigenically different from the first 13 cultures studied.

Acknowledgments

The authors wish to express thanks to Mr. Arturo Riollano for making frequent shipments of diseased cane to the senior author, and to the following workers in the United States Department of Agriculture for hearty cooperation; to Dr. E. W. Brandes, to Mrs. Starrett and Miss Katherine Bryan for making micro-preparations and for photographing, and to Mr. Brewer and Dr. Astchwager for making photographs of the organism. Also to Mr. Arthur F. Bell, of the Bureau of Sugar Experiment Stations of Brisbane, Australia, for sending cultures and photographs for study.

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Explanation of Plates

The photographs were not made on the same scale but the measurements were practically the same in all cases.

Fig. 1.—*B. vascularum.* Photographed by W. Cottrell-Dormer of the Bureau of Sugar Experiment Stations, Brisbane, Australia.

Fig. 2.—The organism from the gumming cane (P.O.J.–2878) at

Jayuya, Puerto Rico. Photographed by A. F. Bell, of the Bureau of Sugar Experiment Stations, Brisbane, Australia, from a culture sent to him by the senior author.

FIG. 3.—*B. vascularum* from white culture from Cristalina from Vieques.

FIG. 4.—*B. vascularum* from yellow culture from Cristalina from Vieques.

NOTE.—Numbers 3 and 4 photographed by Mr. Brewer and Dr. Artschwage of the U. S. Department of Agriculture.

PLATE XXI

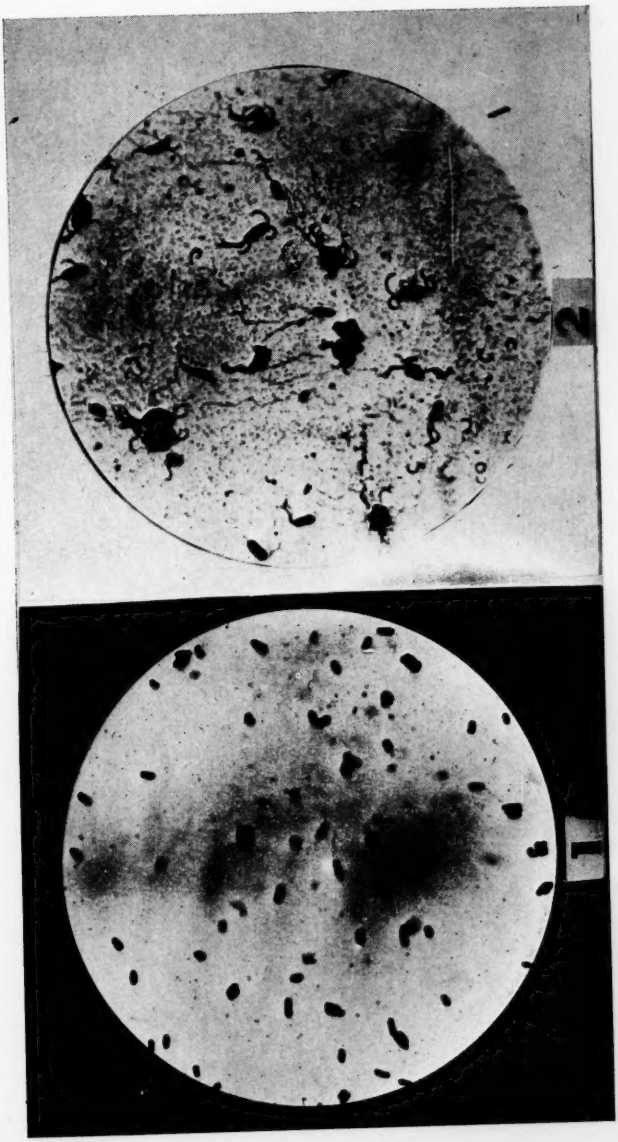

PLATE XXII

RESEARCH ON SHORTENING TIME WITHOUT AFFECTING THE ACCURACY OF DYER'S MODIFIED METHOD FOR THE DETERMINATION IN SOILS, OF PHOSPHORIC ACID, LIME AND POTASH SOLUBLE IN CITRIC ACID SOLUTION (1 PER CENT)

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Since the soil survey of Puerto Rico started in 1928 under the joint cooperation of the U. S. Bureau of Chemistry and Soils and the P. R. Insular Experiment Station, the soil samples sent by the field staff have been analyzed for phosphoric acid, lime and potash soluble in 1 per cent citric acid solution. Before this research was undertaken as one of the projects of the new Soils Division officially created in June 1931, the method used was as follows:

DYER'S MODIFIED METHOD (3)

"Place 100 grams of the air dried soil sample in a Winchester acid bottle. Add 1 liter of a 1 per cent citric acid solution. Shake in a shaking machine 6 hours. Let stand overnight to clear. Filter off about 700 cc of the clear supernatant liquid through a double filter paper. Evaporate exactly 500 cc nearly to dryness in a 600 cc beaker on the hot plate, then transfer the resulting dark colored solution to a platinum dish (using hot water) and evaporate to dryness on a water bath. Completely dry the sticky residue (2 hours in a hot air oven at a temperature of about 120° C.).

"Ignite the residue in the platinum dish in a muffle at low temperature (dull redness) for about 2 hours to remove organic matter. The char should now be gray in color. Moist with con. HCl, add a little hot water and evaporate to dryness on the water bath. Leave on water bath at least 1 hour to dehydrate any silicic acid still remaining as such. Take up in a little con. HCl; add a little water; heat one-half hour on a water bath; add more hot water and filter. Wash five times with hot water slightly acidified with HCl; make up to at least 300 cc with hot water. To the clear filtrate add 2 or 3 cc of con. HNO_3 and boil 15 to 20 minutes to oxidize all organic matter in solution. Precipitate Fe, Al, Ti and P_2O_5 with NH_4OH in

hot solution, filter and wash free from chlorides with hot water. In case of insufficient Fe to color the solution slightly brown, add a few cc of a 10 per cent $FeCl_3$ solution before precipitation. (This is to insure complete ppt. of P_2O_5.)

Phosphoric Acid, P_2O_5

"Dissolve the above ppt. in dil. HNO_3 and boil. Cool slightly, *nearly* neutralize with NH_4OH add 5 grm. NH_4NO_3 and ppt. P_2O_5 with 25 cc molybdate sol. at 65° C. Let stand 20 min. at 65° then let cool. Filter through asbestos, thoroughly wash and det. P_2O_5 volumetrically.

Lime, CaO

"Evaporate down to 150 cc the filtrate from Fe and Al, make very slightly alkaline with NH_4OH and ppt. Ca as oxalate with ammonium oxalate in hot solution. Let stand on the water bath 1 hour then cool over night. Filter, wash with cold water, dissolve in H_2SO_4 (1 to 5 by vol.) and titrate with a standard $KMnO_4$.

Potash, K_2O

"Acidify filtrate from Ca slightly with dil. H_2SO_4. Evaporate to dryness in a beaker, transfer to platinum dish and run to dryness. Carefully ignite off excess of H_2SO_4 and all ammonium salts over a free flame; take up residue with hot water, filter into porcelain evap. dish and add 2 or 3 drops of dil. HCl and sufficient platinic chloride sol. to ppt. all the potassium. Evaporate nearly to dryness, filter, wash and weigh as potassium platinic chloride in the usual gravimetric way."

Experimental

The modified procedures introduced have been as follows:

Place 150 grams of the air dried soil sample in a Winchester acid bottle. Add 1500 cc of a 1 per cent citric acid solution. Shake in a shaking machine, 6 hours. Let stand overnight to clear. Filter.

Phosphoric Acid, P_2O_5

Measure in a beaker, 500 cc of the filtered citric acid extract. Add 10 cc HNO_3 and 30 cc HCl. Evaporate in hot plate, nearly to dryness. Transfer to evaporating dish and dry residue carefully, in sand bath. Ignite the residue at low temperature to destroy the un-

decomposed organic matter and complete the dehydration and destruction of silicates. Dissolve in 5 cc HNO_3 and 15 cc HCl, dilute with water and filter. Follow official molbydate volumetric method. (If a brown color due to manganese persists in the solution after neutralizing with NH_4OH and acidifying with HNO_3, heat to coagulate the colloidal suspension and filter.)

Lime, CaO

Measure in a beaker, 100 cc of the filtered citric acid extract. Determine lime by Chapman's (4) method as follows:

To the solution containing Ca, Mg, Mn, Fe, Al, Ti, and P_2O_5 add about 6 grams of NH_4Cl. One gram of oxalic acid in solution, 10 cc of 1.76 normal acetic acid, and 10 drops of .04 per cent brom cresol green, are added. The solution is made to a volume of about 200 cc and brought nearly to boiling. Dilute ammonia is added slowly until a drop of the solution added to a drop of brom cresol green in a porcelain spot plate changes from yellow thru yellowish green to the first pure green (pH3.9–4.2). The solution is then boiled gently for five minutes, the calcium precipitating in a coarse crystalline state. The precipitate is then allowed to stand on a steam bath until it wholly subsides. Allow the solution to cool before filtration. Wash five times with cold water. Determine CaO by the official permanganate volumetric method.

Potash, K_2O

Evaporate 500 cc of the filtered extract nearly to dryness, on the hot plate. Transfer the resulting dark colored solution to a silica dish (using hot water) and carefully evaporate to dryness on a sand bath. Ignite the residue in a muffle to about 1000° F. (incipient red heat) for about 2 hours to remove organic matter. Add 15 cc HCl, add a little hot water and evaporate to dryness on the water bath. Take up in 5 cc of HCl and water, heat for 30 minutes on a water bath; add more hot water and filter. Wash three times with hot water and make up to about 200 cc. To the clear filtrate add 2 cc of HNO_3 and boil 10 minutes to oxidize all organic matter in solution. In hot solution, precipitate Fe, Al, Ti and P_2O_5 with NH_4OH adding also 25 cc of saturated ammonium oxalate solution to precipitate Ca and Mg. Filter and wash well with hot water. (In case of insufficient Fe to color the solution slightly brown, add a few cc of a 10 per cent $FeCl_3$ solution before precipitation. This is to insure complete precipitation of P_2O_5.)

To filtrate add 2 cc H_2SO_4 (1:1) and evaporate to dryness in sand bath. (It is convenient to add 5 cc of HNO_3 before evaporating to dryness to avoid losses due to the creeping out of the salts.) Ignite to whiteness to expel ammonium salts. Extract with hot water and filter. Determine K_2O by the official Lindo-Cladding method.

Analytical Results of Percent CaO by Chapman's Method

Comparative values by Student's method of percent CaO as determined by Dyer's modified and Chapman's methods are expressed in table I. To eliminate personal error, all analyses by both methods were performed in the same extraction by the same chemist, F. A. Villamil. The letters A or B following a sample number represent duplicate extractions. The values in columns A and B represent percent CaO obtained respectively, by the Dyer modified method and by the Chapman method. The values of column (A–B) are obtained by subtracting the values of column B from those of A. The factor .9702 was obtained by dividing the total of column A by that of B. The values of column C are obtained by multiplying that factor by the values of column B. The values of column (A–C) are obtained by subtracting the values of column C from those of A. The values D and D^1 represent mean deviations. The standard deviation of the mean of column (A–B) is obtained by the formula:

$$S.\ D. = \sqrt{\frac{SmD^2}{n(n-1)}}$$

where SmD^2 is the sum of the values D^2 and n, the number of observations. The standard deviation of the mean of column (A–C) is obtained by that same formula substituting $Sm(D^1)^2$ for SmD^2.

The value Z represents the ratio of the mean of column (A–B) to the square root of the mean of the values D^2. The value (Z^1) represents the ratio of the mean of column (A–C) to the square root of the mean of the values $(D^1)^2$. The statistical interpretation of the results was obtained with values Z and n in the Student's table modified by Love (**9**), $n = 91$ in this case.

TABLE I.

COMPARATIVE VALUES BY STUDENT'S METHOD OF PERCENT CaO AS DETERMINED BY DYER'S MODIFIED AND CHAPMAN'S METHODS.

No.	Dyer's A % CaO	Chapman's B % CaO	A—B	D (A-B+.014)	D^2	C (.9702B)	A—C	D' (A—C—.0004)	$(D')^2$
1	.266	.288	—.022	—.008	.000064	.279	—.013	—.0134	.00017956
2	.697	.731	—.034	—.020	.000400	.709	—.012	—.0124	.00015376
3	.241	.260	—.019	—.005	.000025	.252	—.011	—.0114	.00012996
4	.118	.126	—.008	.006	.000036	.122	—.004	—.0044	.00001936
5	.084	.118	—.034	—.020	.000400	.114	—.030	—.0304	.00092416
6	.051	.064	—.013	.001	.000001	.062	—.011	—.0114	.00012996
7	.518	.529	—.011	.003	.000009	.513	.005	.0046	.00002116
8	.065	.070	—.005	.009	.000081	.068	—.003	—.0034	.00001156
9	.081	.095	—.014	0	0	.092	—.011	—.0114	.00012996
10	.868	.958	—.090	—.076	.005776	.929	—.061	—.0614	.00376996
11	.110	.137	—.027	—.013	.000169	.133	—.023	—.0234	.00054756
12	.087	.098	—.011	.003	.000009	.095	—.008	—.0084	.00007056
13	.378	.403	—.025	—.011	.000121	.391	—.013	—.0134	.00017956
14	.079	.084	—.005	.009	.000081	.081	—.002	—.0024	.00000576
15	.171	.168	.003	.017	.000289	.163	.008	.0076	.00005776
16	.266	.314	—.048	—.034	.001156	.305	—.039	—.0394	.00155236
17	.952	.960	—.008	.006	.000036	.931	.021	.0206	.00042436
18	.554	.596	—.042	—.028	.000784	.578	—.024	—.0244	.00059536
19	.137	.151	—.014	0	0	.147	—.010	—.0104	.00010816
20	.129	.154	—.025	—.011	.000121	.149	—.020	—.0204	.00041616
21	.224	.238	—.014	0	0	.231	—.007	—.0074	.00005476
22	.756	.795	—.039	—.025	.000625	.771	—.015	—.0154	.00023716
23	.286	.305	—.019	—.005	.000025	.296	—.010	—.0104	.00010816
24	.244	.252	—.008	.006	.000036	.244	0	—.0004	.00000016
25	.594	.605	—.011	.003	.000009	.587	.007	.0066	.00004356
26	.532	.546	—.014	0	0	.530	.002	.0016	.00000256
27	.644	.650	—.006	.008	.000064	.631	.013	.0126	.00015876
28	.487	.504	—.017	.003	.000009	.489	—.002	—.0024	.00000576
29	.616	.622	.006	.008	.000064	.603	.013	.0126	.00015876
30	.305	.314	—.009	.005	.000025	.305	0	—.0004	.00000016
31A	.064	.067	—.003	.011	.000121	.065	—.001	—.0014	.00000196
31B	.064	.067	—.003	.011	.000121	.065	.001	—.0014	.00000196
32	.599	.602	—.003	.011	.000121	.584	.015	.0146	.00021316
33	.160	.165	—.005	.009	.000081	.160	0	—.0004	.00000016
34	.132	.134	—.002	.012	.000144	.130	.002	.0016	.00000256
35	.269	.274	—.005	.009	.000081	.266	.003	.0026	.00000676
36	1.310	1.330	—.020	—.006	.000036	1.290	.020	.0196	.00038416
37	.089	.087	.002	.016	.000256	.084	.005	.0046	.00002116
38A	.692	.700	—.008	.006	.000036	.679	.013	.0126	.00015876
38B	.694	.703	—.009	.005	.000025	.682	.012	.0116	.00013456
39A	.694	.683	.011	.025	.000625	.663	.031	.0306	.00093636
39B	.689	.686	.003	.017	.000289	.666	.023	.0226	.00051076
40A	.644	.647	—.003	.011	.000121	.628	.016	.0156	.00024336
40B	.641	.652	—.011	.003	.000009	.633	.008	.0076	.00005776
41A	.549	.554	—.005	.009	.000081	.537	.012	.0116	.00013456
41B	.543	.548	—.005	.009	.000081	.532	.011	.0106	.00011236
42A	.196	.246	—.050	—.036	.001296	.239	—.043	—.0434	.00188356
42B	.196	.241	—.045	—.031	.000961	.234	—.038	—.0384	.00147456
43A	.440	.448	—.008	.006	.000036	.435	.005	.0046	.00002116
43B	.428	.454	—.026	—.012	.000144	.440	—.012	—.0124	.00015376
44A	.336	.353	—.017	—.003	.000009	.342	—.006	—.0064	.00004096
44B	.353	.358	—.005	.009	.000081	.347	.006	.0056	.00003136
45A	2.128	2.134	—.006	.008	.000064	2.070	.058	.0576	.00331776
45B	2.122	2.139	—.017	—.003	.000009	2.075	.047	.0466	.00217156
46A	.428	.437	—.009	.005	.000025	.424	.004	.0036	.00001296
46B	.423	.426	—.003	.011	.000121	.413	.010	.0096	.00009216
47A	.235	.246	—.011	.003	.000009	.239	—.004	—.0044	.00001936
47B	.224	.235	—.011	.003	.000009	.228	—.004	—.0044	.00001936
48A	.333	.336	—.003	.011	.000121	.326	.007	.0066	.00004356
48B	.333	.336	—.003	.011	.000121	.326	.007	.0066	.00004356
49A	.126	.154	—.028	—.014	.000196	.149	—.023	—.0234	.00054756
49B	.157	.143	.009	—.023	.000529	.144	.013	.0126	.00015876
50	.700	.706	—.006	.008	.000064	.685	.015	.0146	.00021316
51	.655	.700	—.045	—.031	.000961	.679	—.024	—.0244	.00059536
52	.865	.888	—.023	—.009	.000081	.862	.003	.0026	.00000676
53	.784	.792	—.008	.006	.000036	.768	.016	.0156	.00024336
54	1.123	1.212	—.089	—.075	.005625	1.176	—.053	—.0534	.00285156
55	.073	.064	.009	.023	.000529	.062	.011	.0106	.00011236
56	.081	.095	—.014	0	0	.092	—.011	—.0114	.00012996
57A	.591	.599	—.008	.006	.000036	.581	.010	.0096	.00009216
57B	.594	.602	—.008	.006	.000036	.584	.010	.0096	.00009216
58A	.406	.412	—.006	.008	.000064	.400	.006	.0056	.00003136
58B	.420	.428	—.008	.006	.000036	.415	.005	.0046	.00002116

TABLE I.

COMPARATIVE VALUES BY STUDENT'S METHOD OF PERCENT CaO AS DETERMINED BY DYER'S MODIFIED AND CHAPMAN'S METHODS—Continued.

No.	Dyer's A % CaO	Chapmans B % CaO	A − B	D (A-B+.014)	D^2	C (.9702B)	A−C	D' (A−C−.0004−)	$(D')^2$
59A	.409	.412	−.003	.011	.000121	.400	.009	.0086	.00007396
59B	.406	.409	−.003	.011	.000121	.397	.009	.0086	.00007396
60A	.185	.193	−.008	.006	.000036	.187	−.002	−.0024	.00000576
60B	.179	.188	−.009	.005	.000025	.182	−.003	−.0034	.00001156
61A	.428	.431	−.003	.011	.000121	.418	010	.0096	.00009216
61B	.428	.431	−.003	.011	.000121	.418	.010	.0096	.00009216
62A	.140	.140	0	0	0	.136	.004	.0036	.00001296
62B	.140	.137	.003	.017	.000289	.133	.007	.0066	.00004356
63A	2.078	2.108	−.030	−.016	.000256	2.045	.033	.0326	.00105276
63B	2.078	2.108	−.030	−.016	.000256	2.045	.033	.0326	.00105276
64A	.081	.104	−.023	−.009	.000081	.101	−.020	−.0204	.00041616
64B	.092	.109	−.017	−.003	.000009	.106	−.014	−.0144	.00020736
65	.107	.118	−.011	.003	.000009	.114	−.007	−.0074	.00005476
66	.132	.134	−.002	.012	.000144	.130	.002	.0016	.00000256
67	.196	.202	−.006	.008	.000064	.196	0	−.0004	.00000016
68	.362	.370	−.008	.006	.000036	.359	.003	.0026	.00000676
69	.070	.070	0	0	0	.068	.002	.0016	.00000256
70	118	.118	0	0	0	114	.004	.0036	.00001296
Sum.	40.752	42.005	−1.253		.025531	40.748	.004		.00307733
Mean	.448	.462	−.014		.00028		.0004		.00034

$$Z = \frac{.014}{\sqrt{.00028}} = .82$$

Odds over 9999:1

$$Z' = \frac{.0004}{\sqrt{.00034}} = .022$$

Odds below 2:1

When both methods are statistically compared, Z=.82 and the odds from the table (**9**) are found to be over 9999:1. The odds indicate a real difference in the analytical results between the two methods; since according to the table odds over 21:1 indicate a significant difference in the results.

The evidence suggests that Chapman's method tends to give higher results with a mean deviation of .014 per cent ± .0017 per cent in 91 determinations or values that may fluctuate between .0123 per cent and .0157 per cent. For comparison purposes the results obtained by either method may be considered entirely satisfactory. That either is as good as the other is corroborated by the excellent checks obtained by each method in the duplicate of different extractions for samples 31, 38–49 inclusive, and 57–64 inclusive. The statistical interpretation, therefore, does not invalidate at all, Chapman's method.

Chances for personal analytical error are much less in the Chapman method since the work is greatly facilitated by the elimination of the cumbersome procedure required for the precipitation of Al, Ti, and P_2O_5 with NH_4OH in the Dyer modified method. The former method saves about 66 per cent of the time required by the latter.

Were it necessary to convert results obtained by Chapman's

method to those in terms of the Dyer modified method, it would be proper to multiply by the factor .9702. The value Z^1, after the application of such a factor to each result obtained by Chapman's method becomes .022, and the odds from the table are found to be below 2:1. The odds indicate that there is no significant difference between the analytical results of both methods. Results indicate that the increase by Chapman's method tends to be constant. The mean deviation becomes now, .0004 per cent ± .0017 per cent or a fluctuation between —.0013 per cent and .0021 per cent which is extremely low.

Colorimetric Methods for Phosphoric Acid

Studies were made of the molybdate blue method with the modifications of Briggs and Doisy applied by Arrhenius (1) to citric acid extracts. The method is as follows:

"Ten cc of the citric acid extract are placed in a 100 cc volumetric flask and diluted to about 80 cc; 1 cc con. H_2SO_4, 5 cc ammonium molybdate (25 gms. ammonium molybdate dissolved in 300 cc. water) and then 200 cc dilute sulphuric acid (75 cc conc. H_2SO_4 filled up to 200 cc with water), 1 cc sodium sulphite (20 g. Na_2SO_3 + 80 cc water), and 1 cc hydroquinone (0.5 g. per 100 cc and one drop conc. sulphuric acid), are added. The flask is filled to the mark and the solution shaken. The color is, after 12 to 24 hours, compared with a standard series of solutions with known P_2O_5 content varying between .05 and .90 mgm. P_2O_5 per 100 cc." The so-called Arrhenius molybdate blue method for P_2O_5 determinations in citric acid extract is based on the one recommended by Bell-Doisy-Briggs (2) for P_2O_5 determinations in urine and blood, with the exception that the addition of trichloracetic acid for the precipitation of protein material is eliminated.

Although Arrhenius claims that results obtained are accurate below 0.50 mgm. P_2O_5 (.05 per cent P_2O_5 in the 10 cc citric acid aliquot used by us), our results were entirely inconsistent. The citrate ion colored by the presence of iron salts causes difficulty in matching the colors of the unknown and the standard in the colorimeter.

Studies were made of the method that Warren and Pugh (11) worked out at the Rothamsted Experimental Station based on the colorimetric determination of phosphoric acid in citric acid extractions of soils as follows:

"Seventy-five cc of the citric acid extract are pipetted into a 300 cc Kjeldahl flask, 10 cc conc. HCl added and followed by 12 cc of 20 per cent sodium permanganate. The sides of the flask are washed

down with a little water. After standing half an hour the contents are vigorously digested till no manganese precipitate remains (about ½ hr. more). The contents are transferred with a minimum amount of water to a 100 cc graduated flask; 4 cc of 10 per cent potassium ferrocyanide are added slowly, drop by drop, with frequent shaking. Several minutes later the mixture is titrated with 1:1 ammonia until the blue color just turns purple; 1.5 cc 2 N sulphuric acid are then added and made to the mark with water. After the solution has been filtered and the first few cc discarded, the color is developed in an aliquot by one of the following methods:

Fiske—Subbarrow (**6**) "10 to 50 cc are pipetted into a 100 cc graduated flask, diluted to 75 cc approximately, 10 cc of ammonium molybdate added, then 4 cc aminonaphthol sulphonic acid solution and the liquid made to the mark. The flask should be shaken during each addition. The contents are finally poured into a 100 cc conical flask; 15 minutes later the test compared with a standard phosphate solution".

Deniges (**5**)—"1 to 25 cc are pipetted into a 100 cc graduated flask, diluted to 90 cc, and 1 cc ammonium molybdate and three drops of stannous chloride solution added, the flask being shaken with each addition. After diluting to the mark the contents are poured into a 100 cc conical flask, and compared after 5 minutes with a standard phosphate solution".

Our results with the Warren and Pugh method were extremely low compared with those obtained by the usual Dyer method. This may be due to the adsorption of phosphates by the manganese ferrocyanide precipitate. Ward (**10**) states that "the ferrocyanide precipitate is difficult to filter, because of its colloidal condition and that the amount of phosphorous present in the sodium permanganate is of the same order of magnitude as that in the sample, and inaccurate results are certain to follow in the case of soils low in P_2O_5".

Lonstein (**8**) applied the method of Deniges, so widely used for water extracts, to citric acid extracts of a number of South African soils and obtained good agreement with the gravimetric method. His method briefly consists in evaporating a small volume of the citric acid extract to dryness after the addition of calcium acetate solution. The residue is ignited to destroy the organic matter and to render the silica insoluble, and then extracted with 10 per cent sulphuric acid. After filtration the excess of the acid is neutralized with ammonia and the color developed with ammonium molybdate and stannous chloride solutions.

Although we were favorably impressed by the Lonstein method we disregarded it because the figures obtained with it by Warren and Pugh (**11**) "reveal the presence of some disturbing factor in the analyses of the heavier soils used, especially when the Deniges method

of color production was used. The blue color developed slowly and had a green tint. The view was sustained that the sulphuric acid extracts from the clay soils contained appreciable amounts of ferric iron as the interfering constituents''.

One of the latest contributions to the subject on the colorimetric determination of phosphorus in citric acid extracts of soils is the work of Ward (**10**), chemist of the Experiment Station of the Hawaiian Sugar Planters' Association. Ward's procedure for the preparation of the citric acid extract is as follows: ''To 100 ml. of citric acid extract, add 50 ml. of concentrated nitric acid, 15 ml. of concentrated hydrochloric acid, and 10 ml. of 20 per cent sulfuric acid free from phosphorus and arsenic. Evaporate slowly till fumes of SO_3 are evolved. Take up in hot water and boil''. The iron is removed by electrodialysis into a special electrolytic cell. The color is developed by the method of Zinzadze as follows: ''Aliquots from the solutions of citric acid extract prepared for analysis are neutralized to the yellow end point of alpha—dinitrophenol indicator with 10 per cent ammonia solution. Dilute the solutions to 90 ml., add 1.4 ml. of molybdenum blue reagent (molybdic acid reduced by molybdenum metal in sulphuric acid solution), heat for 30 minutes on the steam bath, cool, and make up to exactly 100 ml. Employ a sensitive colorimeter for comparison with the standard solution''.

The work of different investigators reveal that the accurate colorimetric determinations of phosphoric acid in soils extracted with 1 per cent citric acid, demands attention on the destruction of the citrate ion, and of the soluble silicates and organic matter; the absence of large amounts of silica; the absence of phosphorous in the chemical reagents used; the elimination of the ferric ion; and a controlled acidity. Ward (**10**) calls the attention to the presence of titanium and to the use of colorimetric standards which are very close to the unknown in color intensity so as to reduce errors due to deviations from Beer's (**12**) law.

Treatments of the Citric Acid Extracts for the Phosphoric Acid Determinations

In view of the several difficulties that we encountered in obtaining accurate results with the application of colorimetric methods for the determinations of phosphoric acid in soils extracted with 1 per cent citric acid we proceeded to study the simplification of the chemical treatments given to such extracts in the Dyer modified method and then followed the official molybdate volumetric method for phosphoric acid.

Our first attempt was to precipitate directly phosphoric acid as ammonium phosphomolybdate in aliquots of the citric acid extracts. In some cases, good checks were obtained with the results by the Dyer modified method. In other cases, no precipitate was obtained; and in other cases, lower results were obtained. This may be explained on the basis that citric acid, extracts from certain soils, phosphates in organic combinations that are not precipitated by ammonium molybdate, and that the presence of the undecomposed soluble silicates may hold by absorption the phosphate ions in solution.

Our second attempt was to destroy the organic and silicate compounds by evaporation almost to dryness, with aqua regia. The residue was taken with a few cc of aqua regia, diluted with water, and filtered. Phosphoric acid was determined in the filtrate by the official molybdate volumetric method. Although our results checked much better with those obtained by the Dyer modified method we were unable to obtain the ammonium phosphomolybdate precipitate in several of the treated extracts. It seems that the aqua regia treatment was not drastic enough, in some cases, to decompose some of the silicates that would tend to establish a competition for the adsorption of the phosphate ions in solution.

Our next procedure was then to evaporate the citric acid extracts to dryness with aqua regia and ignite the residue at low temperature to destroy the undecomposed organic matter and complete the dehydration and destruction of silicates. This treatment served as a basis for our recommended procedure (see pp. 288–289). Analytical results are expressed in Table II.

Analytical Results of Percent P_2O_5 by our Short Procedure

All analyses by both methods were performed in the same extraction. The letters A and B following a sample number represent duplicate extractions. All the analyses by Dyer's modified method and by our short procedure in samples 18, 20 and 23–27 inclusive, were made by the same chemist, F. A. Villamil. All other analyses by our short procedure were made by the senior author. The values in columns A and B represent percent P_2O_5 obtained respectively by the Dyer modified method and by our short procedure. The values of column A–B are obtained by subtracting the values of column B from those of A. The values D represent the mean deviations. The number of determinations $n = 45$. The value Z represents the ratio of the mean of columns (A–B) to the square root of the mean of the values D^2. The statistical interpretation of the results were obtained with values Z and n in the Student's table modified by Love (**9**).

TABLE II.

COMPARATIVE VALUES BY STUDENT'S METHOD OF PERCENT P_2O_5 AS DETERMINED BY THE DYER MODIFIED METHOD AND OUR SHORT PROCEDURE

No.	Dyer's A % P_2O_5	Short Procedure B % P_2O_5	A—B	D (A—B—.0003)	D^2
1A	.014	.016	—.002	—.0023	.0000529
1B	.014	.015	—.001	.0013	.00000169
2A	.010	.009	.001	.0007	.00000049
2B	.010	.009	.001	.0007	.00000049
3A	.008	.008	0	—.0003	.00000009
3B	.008	.008	0	—.0003	.00000009
4A	.007	.007	0	—.0003	.00000009
4B	.007	.007	0	—.0003	.00000009
5A	.013	.011	.002	.0017	.00000289
5B	.013	.010	.003	.0027	.00000729
6A	.005	.005	0	—.0003	.00000009
6B	.004	.006	—.002	—.0023	.00000529
7A	.002	.004	—.002	—.0023	.00000529
7B	.002	.005	—.003	—.0033	.00001089
8A	.017	.017	0	—.0003	.00000009
8B	.015	.017	—.002	—.0023	.00000529
9A	.006	.008	—.002	—.0023	.00000529
9B	.007	.006	.001	.0007	.00000049
10	.018	.017	.001	.0007	.00000049
11A	.036	.039	—.003	—.0033	.00001089
11B	.036	.039	—.003	—.0033	.00001089
12A	.146	.148	—.002	—.0023	.00000529
12B	.145	.155	—.010	—.0103	.00010609
13A	.023	.015	.008	.0077	.00005929
13B	.023	.014	.009	.0087	.00007569
14A	.013	.013	0	—.0003	.00000009
14B	.013	.013	0	—.0003	.00000009
15A	.019	.016	.003	.0027	.00000729
15B	.019	.016	.003	.0027	.00000729
16A	.023	.025	—.002	—.0023	.00000529
16B	.023	.025	—.002	—.0023	.00000529
17	.003	.002	.001	.0007	.00000049
18	.023	.020	.003	.0027	.00000729
19A	.052	.054	—.002	—.0023	.00000529
19B	.051	.053	—.002	—.0023	.00000529
20	.048	.053	—.005	—.0053	.00002809
21A	.019	.013	.006	.0057	.00003249
21B	.018	.013	.005	.0047	.00002209
22A	.005	.004	.001	.0007	.00000049
22B	.006	.004	.002	.0017	.00000289
23	.004	.001	.003	.0027	.00000729
24	.008	.005	.003	.0027	.00000729
25	.007	.005	.002	.0017	.00000289
26	.002	.001	.001	.0007	.00000049
27	.004	.002	.002	.0017	.00000289
Sum	.924	.908	.016		.00047045
Mean	.0205	.0202	.0003		.0000105

$$Z = \frac{.0003}{\sqrt{.0000105}} = .09$$

Odds: Below 3:1

When both methods are statistically compared, $Z = .09$ and the odds from the table (**9**) are found to be below 3:1. The odds indicate that there is no significant difference between the analytical results obtained by both methods.

That either method is as good as the other is corroborated by the excellent checks of the duplicates in different extractions.

Chances for personal analytical error are much less in the short

procedure since the work is greatly facilitated by the elimination of the cumbersome procedure required for precipitation of Fe, Al, Ti and P_2O_5 with NH_4OH in the Dyer modified method. The former method saves about 30 per cent of the time required by the latter.

Potash

Several modifications for the Dyer modified method were tried. Our results were unsatisfactory. We recommend the Dyer modified method with the following modification: Precipitate the lime together with the iro nand phosphoric acid. (See pp. 289–290).

Through the courtesy of Dr. W. W. G. Moir, agricultural technologist of the American Factors, Limited, Hawaii, and L. E. Davis, associate chemist of the Experiment Station of the Hawaiian Sugar Planters' Association, we obtained a reprint of Gow's (7) work on a rapid colorimetric method for the determination of potash. "This method consists essentially of precipitating potassium chloroplatinate by means of its insolubility in alcohol, dissolving the precipitate in water and developing a color by the addition of stannous chloride. The intensity of the yellow color thus produced is directly proportional to the amount of platinum present in the precipitate and hence to the amount of potash present."

SUMMARY

Research was undertaken with the purpose of shortening time without affecting accuracy of the Dyer modified method for the determination in soils, of phosphoric acid, lime and potash soluble in 1 per cent citric acid solution.

A short method is recommended for the lime determination based on the Chapman's method (4) for the precipitation of calcium oxalate in acid solutions (pH 3.9–4.2) in the presence of iron, aluminum, titanium, manganese, magnesium and phosphates. A short method is recommended for the phosphoric acid determination. Results are analyzed statistically by Student's method. Several of the colorimetric methods recommended for the phosphoric acid determination are also discussed.

Acknowledgments

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G. Capó, analytical chemist for the fertilizer control laboratory of the Department of Agriculture and Commerce and to Mr. Fernando Chardón, tobacco specialist of the Phytotecnics Division, Insular Experiment Station for valuable suggestions relative to the statistical interpretation of the chemical data, and to Dr. W. W. G. Moir, agricultural technologist of the American Factors, Limited, Hawaii and L. E. Davis, associate chemist of the Experiment Station of the Hawaiian Sugar Planters' Association for their courtesy in sending reprints of references (**7**) and (**10**), and for the proper citation of reference (**3**) which we requested after being unable to obtain the bulletin referred.

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A VARIETY OF TOBACCO RESISTANT TO ORDINARY TOBACCO MOSAIC

By J. A. B. Nolla, John Simon Guggenheim Memorial Foundation Fellow, 1932–33, University of Wisconsin; and Arturo Roque, Assistant Phytopathogist, Insular Experiment Station, Puerto Rico.

The ordinary mosaic of tobacco affects a wide range of solanaceous hosts including most members of the genus Nicotiana. It is particularly severe on all varieties of *Nicotiana tabacum* L. heretofore studied in relation to the disease. In spite of the common occurrence of this virus disease on *N. tabacum* and the unusual opportunity afforded for the recognition of varietal or strain resistance to the disease, no such development has apparently been reported. Furthermore, the economic aspects of control of this malady are of such a nature that plant pathologists must have been on the lookout for cases of resistance to the disease for many years.

In the summer of 1929 the senior author visited the Cauca Valley of Columbia, South America. In these trips he observed several varieties of commercial tobacco, and his interest was curiously drawn to two varieties, seed of which was introduced into Puerto Rico. This seed obtained from Señor Carlos Rivera G., of Palmira, Department of Valle del Cauca, was presumably from the same stock of which plants were seen grown at the Santa Ana Farm of the Compañía Colombiana de Tabaco. The exact location is near the southeastern limits of the Department of Valle del Cauca.

The two varieties were grown in Puerto Rico, near Caguas, in 1929–1930 and it was during that year that the senior author observed the almost complete freedom from mosaic in one of the varieties, namely, the *Ambalema*. In a population of some six hundred plants, four individuals showed the disease as evidenced by a slower growth and by the characteristic symptoms on leaves. Unfortunately, no further studies were made with the susceptible plants. Selection of resistant plants was made from that progeny and crosses between these and a susceptible variety begun that year.

The senior author left Puerto Rico in the summer of 1930 and his studies were delayed for one year while doing graduate studies at Cornell University. He resumed this investigation in the fall of 1931.

In the meantime, the junior author, working at the Insular Experiment Station of Puerto Rico, discovered, independently, the resistance of this variety of tobacco to mosaic.

Both authors are now engaged in a study of the inheritance of resistance to mosaic. Our observations show that most plants of this variety inoculated with the virus of the ordinary tobacco mosaic do not exhibit any very marked symptoms. However, a clearing of the veins may be noticed in some individuals, while a smaller percentage exhibit chlorotic areas of a mild type, in the intervenal regions of the blade of the leaf.

Since the genetic nature of the resistance of this variety of tobacco was not well understood, an earlier note had seemed unwarranted. An explanation for the cases of susceptible individuals would have been desirable before publishing any note on resistance of the variety to mosaic. The senior author, in his annual report to the Chief Pathologist [See Cook (**2**)] in 1930, said concerning this variety:

. . . . "of the Colombian varieties one proved to be very promising", Page 97. Quite intentionally no mention is made of any specific quality.

Knowledge that the planting of this variety of tobacco is being extended in some places of the Island has prompted the writers to release this preliminary note. We wish to make it clear at this time that we are not responsible for the distribution among farmers of a variety of tobacco which has not yet been submitted to adequate selection and testing.

The senior author wishes to call attention to the fact that at the time the tobacco was collected in Colombia, no observations of resistance to mosaic were made. Our notes on prevalence of mosaic on the various farms rather showed that the variety was apparently susceptible. That we failed to detect freedom from symptoms of mosaic at that time may be explained in two ways. Either the mosaic of tobacco of that part of the continent is more virulent than the mosaic of Puerto Rico and the United States; or, as is most probable, the plants represented a very heterozygous population in which the susceptible individuals predominated. It is of interest, that in a small planting in Toro, Colombia, inspected on June 11th, no mosaic was observed even in plants a year old. No particular significance was attached to this observation at that time, and it was believed that such freedom from mosaic was the result of good cleaning of seed and careful handling of beds and seedlings.

The first illustrative record of this variety of tobacco, as far as the authors know, appeared in 1930 in a Puerto Rican publication (**3**). That picture was taken by the senior author near Toro, on June 11th, 1929. This town is located in the northern part of the Depart-

ment of Valle del Cauca. In this locality, however, the name given to the variety is "Pájaro Negro" (black bird). A plant of the first Puerto Rican grown tobacco of this variety is illustrated opposite page 97, Annual Report of the Insular Experiment Station of Puerto Rico, 1929–30. Bunker (1) publishes a figure of a "Variedad Colombiana Seleccionada y Aclimatada por el Especialista . . ." (Colombian variety selected and acclimated by the Specialist) . . ." Bunker obtained seed from our experimental plots of 1929–30 and, therefore, his plant must have been photographed the following season.

Doctor Carlos Durán Castro, formerly Director of the Agricultural Station at Palmira and now Chief of the Department of Agriculture in the Ministerio Nacional de Industrias at Bogotá, accompanied the senior author in most of his trips, being a source of constant encouragement. His courteousness on the one hand, and his devotion to our work, on the other, more than compensated for our efforts. He should have a considerable share of whatever credit may be reaped from the discovery of this important tobacco variety. The Hon. Carlos E. Chardón, formerly Commissioner of Agriculture of Puerto Rico and now Chancellor of the University of the Island, head of the Agricultural Mission to Colombia in the summer of 1929, also deserves our recognition for his valuable cooperation.

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THE PINEAPPLE DISEASE OF SUGAR CANE IN PUERTO RICO

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This disease is caused by *Thielaviopsis paradoxa* (De Seynes) von Höhn and is widely distributed throughout the cane-growing parts of the world. This fungus was found first on pineapple in 1886 by De Seynes, who described it under the name of *Sporochisma paradoxum*. In 1892 Saccardo referred it to *Chalara paradoxa* (De Seynes) Sacc. In 1893 Went reported *Thielaviopsis ethaceticus* as the cause of a serious disease of sugar cane in Java. In 1904 von Höhnel reported that the organisms described by De Seynes and by Went were the same and made the combination *Thielaviopsis paradoxa* which has been very generally accepted from that time to the present. Dade (1928) (**4**) reported the finding of a *Ceratostomella* which he believed to be the perfect stage of the fungus which would become *Ceratostomella paradoxa*.

The fungus has been reported as attacking a large number of species of plants—sugar cane, pineapple, plantain, mango, coconut, date palm and many others. It has been reported from nearly all the countries in which sugar cane is grown. The geographical distribution according to the International Survey of the Disease of Sugar Cane (**3**) with a few additions, is as follows: Argentina, Australia, British Guiana, Ceylon, Colombia, Cuba, Dutch East Indies, Fiji, Formosa, Gold Coast, Hawaii, India, Jamaica, Java, Lesser Antilles, Madeira, Mauritius, Malaya, Mexico, Philippines, Portuguese East Africa, Puerto Rico, Reunion, Santo Domingo and Southern United States. It is reasonable to suppose that it occurs in all the other sugar-cane-growing countries. In Puerto Rico this fungus attacks the pineapples and is sometimes the cause of heavy losses in fruits and in young slips which have been planted for a short time. It attacks the coconuts and is sometimes the cause of the falling of a large number of young fruits and sometimes attacks the buds, causing a deformity of the young leaves and a slow dying of the trees. It attacks sugar cane, causes a rotting of seed cuttings and poor germinations, which will be described in this paper.

Went (1893) in the first record of this fungus attacking sugar cane stated that it was the cause of a serious disease. Some of the

records since that time have indicated that it was serious while others have indicated that it was of little or no importance. Johnston (7) conducted experiments in Puerto Rico which were published in 1913 for the purpose of determining the relative resistance of a number of varieties. In these experiments he punched a hole in the end of the seed cutting and injected the fungus with a 10 cc syringe. He reports injuries ranging from 15 to 90 per cent but none of the varieties used by him are of commercial importance on the island at this time. He also conducted experiments for the control of the disease and reported that best results were obtained by treating seed cuttings with 4–5–50 Bordeaux and by sealing the cut ends with tar.

When the writer came to Puerto Rico in 1923, inquiries were made concerning the problems of germination. All reports indicated that poor germination was rare and that when it did occur that it was due to poor seed cuttings. Other problems required the time of the writer and no further consideration was given to the problem of poor germination until the spring of 1930 when our attention was called to very poor germination in one locality. A personal examination showed that the soil was heavy and poorly drained and that the seed cuttings were rotting as a result of attacks of *Thielaviopsis paradoxa*. Experiments were started on a small plot of ground at the Experiment Station and have been conducted over a period of three years.

The method consisted in the planting of fifty seed cuttings each of some of our most important varieties, using pieces with three eyes and allowing them to grow for six to twelve weeks. At the end of the time, they were lifted and the germinating eyes counted. In order to make sure of uniform inoculations, the fungus was grown in large flasks and put into a bucket of water. The canes were dipped in this mixture just before planting. After a time, the soil was so thoroughly infested that this was unnecessary. This was demonstrated by treating one half of the cuttings in one planting. When the young plants were lifted and the germinating eyes counted, the results were practically the same for the treated and untreated cuttings. Eleven crops were grown. The soil became so heavily infested as a result of repeated use for this work that the germination of the last three crops was very low. The varieties used were B.H.–10(12), S.C.–12(4), Uba, P.R.–801, 803, 807, 809, 820, 826, Co.–281, F.C.–916, P.O.J.–2714, 2725, 2727 and 2878. The work has not been altogether satisfactory because it was impossible to control the environmental factors in field experiments. One planting was damaged by the overflow of the river and the data very unreliable. However, the results are of some value. These results may be summarized as follows:

1. The so-called pineapple disease of sugar cane in Puerto Rico which is caused by a fungus (*Thielaviopsis paradoxa*) is occasionally very severe.

2. It is most severe on heavy, clay, poorly drained soils.

3. It is most severe during the cooler months of the year.

4. During the summer months the fungus appears to be more abundant or more active in the higher elevations (up to 2000 ft.) than in the coastal plains.

5. In heavily infected soil the rotting of seed cuttings may be as great during dry as during rainy weather, if other conditions are favorable.

6. With two exceptions, the germination tests were below 50 per cent. Both exceptions were with Co.–281. The average germination in Puerto Rico is about 70 or 75 per cent according to experienced cane growers who have been consulted by the writer.

7. Co.–281 proved to be most resistant with other varieties in the following order: P.O.J.–2878, P.R.–801, P.R.–803, P.R.–826, Uba, B. H.–10(12), P.R.–820, P.R.–807, F.C.–916, P.O.J.–2725, S.C.–12(4), P.O.J.–2714, P..R.–809, P.O.J.–2727.

Symptoms

The fungus has been recognized as a wound parasite for many years. It penetrates the cut ends of the seed pieces and destroys the cell walls of the parenchyma tissues. The first evidence of an attack by this fungus is a reddish discoloration of the tissues, followed by a black color and by decay. The decay is accompanied by an odor which is very similar to that of pineapples which are rotted by this fungus. When the decay is well advanced, if one will cut through the rind and break a cane the fibro-vascular bundles can be pulled out in mass like the hairs of a brush.

No studies were made on the temperature relations but Klotz and Fawcett (8) state that the optimum temperature for this fungus on the date palm is 24 to 27½ degrees C. This is approximately 64.5 to 67.5 degrees F. which is a little lower than the temperature of our coastal plains during the winter months.

Treatments

Severe outbreaks of this disease in Puerto Rico are so rare that it is not necessary to use a treatment very often. However, severe outbreaks are likely to occur at any time on poorly drained soils, es-

pecially during the winter months. These outbreaks are expensive in proportion as they make replanting necessary. The first and most important treatment is a well-drained soil. In case of heavily infected soil it may be desirable to plant some other crop for a season or to use the Bordeaux treatment. This must be decided by the grower on basis of farm management and economics. In case the Bordeaux treatment is used, the cane should be treated very soon after cutting. If the seed cuttings become infected before treatment, the treatment is useless. When the grower has a few cuttings of a new variety, it is advisable to protect them by dipping the cut ends in tar or melted paraffin.

The Three-bud Seed Piece

The Puerto Rican practice is to use three-bud seed cuttings. This practice is no doubt the result of long years of experience and the writer believes that the *T. paradoxa* has been the deciding factor in favor of this method. Experimental work by the writer and others has demonstrated that there is a higher germination with three-bud cuttings than with cuttings of two buds or one bud. This is probably due to the length of the seed pieces rather than to the number of buds. In a long seed cutting the fungus has to travel a much longer distance than in short seed pieces and the buds have a better chance to germinate and become established before the fungus reaches them. The writer has found that when short seed cuttings for experimental studies are planted in unsterilized soil, it is advisable to dip the cut ends in melted paraffin or tar.

Other Causes of Poor Germination

Marasmius sacchari is one of the minor causes of poor germination in Puerto Rico. In one planting of one variety, this fungus killed 20 per cent of the buds.

Other causes of poor germination that have been reported from other countries are *Colletotrichum falcatum* and *Melanconium sacchari* in Louisiana, *Ceratostomella adiposum* in India and *Lasiodiplodia theobromae* in the Philippine Islands.

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WHITE SPOT OF PINEAPPLES

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This disease was reported from Puerto Rico by G. L. Fawcett of the Porto Rico Agricultural Experiment Station (Mayagüez) in 1908, who described it as follows:

"The pineapple has one conspicuous disease characterized by the appearance of white spots on the leaves. This is caused in many cases at least by the entrance of organisms through wounds such as the punctures made by sucking insects. At first the spots are small and brown. Gradually they enlarge, the epidermis sinks, the interior tissue is destroyed, and the white bleached appearance is taken on. It is in no sense a serious disease although common under excessively moist conditions."

Nowell (1922) says:

"A leaf-spot which is fairly common in the West Indies has been investigated by L. D. Larsen in Hawaii. It occurs on well-grown plants and its effect on their development is not usually appreciable. It has been shown to be due to the infection of insect and spine punctures, or other injuries, by the fungus Thielaviopsis. Infection and development depend on the prevalence of moist and cloudy weather or on the shading of the plants."

Larsen of Hawaii published a bulletin on diseases of pineapples in 1910 in which he described what appears to be the same disease as follows:

"The spots vary considerably, as regards size, shape and color. Many are large and white, and are noticeable from along distance, while others are small and inconspicuous. In typical mature spots there is a straw colored central area surrounded by a dark margin. Very often there is a dark center within the straw colored area, or dark blotches, due to the formation of the black macrospores within the tissue, may be scattered over it in an irregular manner. Sometimes long white arms extend beyond the dark border, and, again, the entire spot may be white or straw colored throughout. The internal tissue is soft and decayed at first, but this soon dries out, leaving the injured area dry and shrunken. The white or straw-colored area is not due simply to drying of the affected tissue, as is often the case with similar diseases, but comes on while the tissue is still quite firm, and long before it has begun to dry out. In early stages the spots may be olive brown in color and fairly regular in outline, or they may be white and irregular from the start. The size of the spots increases very rapidly, so that in two or three days what was a small brown spot will have become from two to six inches in length. Even twelve inches is not an unusual length."

Larsen believed these spots to be due to *Thielaviopsis paradoxa* which gained entrance to the plant through slight wounds. He said:

"The fungus, Thielaviopsis, which as we have seen causes Soft Rot of the pineaple fruit and Base Rot of the cuttings, is also responsible for a conspicuous disease of the leaves of this plant. It attacks the leaves through surface wounds made by insects and by the spines and sharp edges of neighboring leaves, and produces large discolorations or spots which often spread across the entire leaf and thereby kill all that part of the leaf which is above the spot. The spots may be found in almost any of our pineapple fields during the winter months, and at times become exceedingly numerous. They are most prevalent in the finest fields where the plants are green and vigorous, while in poor fields and on yellow plants they are more rare."

He conducted experimental work on which this conclusion was based and which demonstrated that the fungus could not gain entrance to the plant except through wounds. He also made very interesting studies on the influence of weather which he described as follows:

"In the infection experiments conducted it was found that when inoculations were made in the presence of bright sunlight, or if the leaves were exposed to sunny and dry weather conditions on the following day, but very few of the inoculations would take. It was therefore necessary when inoculating leaves under such conditions to cover the wounds with grafting wax or paraffin. When the plants were shaded or when cloudy weather existed after inoculation, infection took place very readily without such protection. Direct sunlight prevents infection; first by drying out the wounds, and secondly, by killing the spores of the fungus. In the experiment mentioned on page 15 it was found that the most resistant form of Thielaviopsis spores are killed in a few hours when exposed to direct sunlight. With this in view it is evident why the spots are scarcely known during the dry summer season and yet become very numerous in the winter months.

"The size of the spots is also governed largely by weather conditions. Sunlight and dry weather, after infection has taken place, tend to limit the size while moist and cloudy weather has the opposite effect. In our infection experiments when the plants were well shaded the entire leaf would often become involved.

"It was furthermore noted that the typical white condition would make its appearance only on leaves that were exposed to sunlight. When inoculated plants were kept in the shade the spots would invariably be brown in color and very regular in outline, whereas when exposed to sunlight the white and irregular patches would appear. They appeared, however, in a sporadic manner, occurring on one leaf here and another there, while other leaves inoculated at the same time and equally exposed would show none of it. It was at first thought that this white appearance was a distinct trouble due to some cause other than Thielaviopsis, but judging from the results of our infection experiments such does not appear to be the case."

A very pronounced outbreak of this disease in the vicinity of Co-

rozal was called to the attention of the writer in August of 1932. These plantings were at an elevation of about 350 or 375 feet and on the boundary line between limestone soils of the coastal plain and the volcanic acid soils.

There had been heavy rainfall and the disease appeared in such abundance that the grower became very much alarmed. The fungus (*Thielaviopsis paradoxa*), which is well known as a parasite on a number of species of plants in Puerto Rico, had been reported previously to the writer as the cause of a very serious fruit rot in this locality. The symptoms, the weather conditions and the presence of the fungus, conformed with Larsen's Hawaiian report of 1910.

A large amount of the diseased leaves were collected and taken to the laboratory for study. A study of free-hand sections did not show a fungus. A large number of cultures were made but the fungus did not develop in any of them. *Thielaviopsis paradoxa* grows readily in culture and the writer has isolated it many times from decaying pineapple fruits, slips, sugar cane, coconuts and other plants, using the same medium as was used at this time. Material was then prepared for paraffing sectioning and staining but the results were again negative. It was impossible to conduct inoculation work at the Experiment Station, owing to lack of plants.

When the rainfall was reduced and bright sunshine prevailed for a few days, no new spots were formed. This was followed by another period of rainy weather and another outbreak of the disease. Therefore, it appears from these studies that these spots may be the results of weather conditions without the influence of any other agency. Field studies by the writer did not indicate that a puncture by a spine, insect or any other agency was necessary for the formation of these spots.

PATHO-ANATOMY OF ROOTS ATTACKED BY NEMATODES

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Very little study has been made in America of the anatomy of plants attacked by nematodes since the publication of Atkinson's "Nematode Root-Galls" (1) in 1889. During the past few years the writer has devoted some attention to this subject and the data on the dicotyledonous plants will be presented in this paper. The plants used in these studies were tomato, tobacco and ornamental Coleus. The results were the same in all cases and it would have been impossible to distinguish them except for the labels. The galls were caused by *Heterodera marioni* (Cornu) Goodey (= *Heterodera radicicola* Greeff).

The nematodes attack the very young roots. The writer is of the opinion that they rarely, if ever, attack roots more than one millimeter in diameter. Young roots contain a large amount of tissue that is capable of division when stimulated. This includes the cambium, cortex and medullary rays. The parasites penetrate into the cortex and the rays where they stimulate an excessive growth. The writer is of the opinion that they rarely if ever penetrate into the fibrous or tracheary tissue.

All cells that retain the power of division are stimulated by the parasites. This stimulation results in the formation of a large number of very small cells in close contact with the parasite while those a distance are much larger (Figs. 3 & 5b). When the nematode attains full size the stimulation appears to cease and the cells become larger (Fig. 4b). The parasite does not appear to stimulate cell division or growth in the fibrous or tracheary tissues, but does push them out of the normal position. The parasite also causes the formation of tracheary tissues from and in the parenchyma tissue (Fig. 1b.)

Nematodes in the axis cylinder (Fig. 2) penetrated through the parenchyma cells and not through the fibrous or tracheary cells.

The writer is inclined to believe that galls may be reinfected many times and that reinfection accounts for the larger galls. The tissues of a gall are highly meristematic as a result of stimulation by previous infections and are very favorable for reinfections. The tissues of the large galls are in such a state of confusion resulting from the large number of parasites and the reinfections that a histological

study is of little or no value. Any satisfactory study of the development of these galls should begin with the very young galls.

Atkinson's studies evidently were made on the galls that were advanced to a point where it was impossible to determine the incipient characters, but his description and discussion are very interesting. He said:

"The worms locate preparatory to passing into the cystic state at various depths in the tissues of the root. They are not confined to any particular tissue element or system but locate in the vascular tissue of the central cylinder, the cambium, parenchyma or even in the bark so that the body of the mature female cyst is frequently only protected by a thin layer of the dead peripheral tissue, or sometimes is even exposed. They seem to flourish better, however in or near the softer tissues of the root. It is a very common thing to find dead undeveloped female cysts, the majority of which I have always found in the woody tissue of the central cylinder. Possibly surrounded as they are by the harder, more compact tissue there is less certainty of the male reaching them for fertilization. This, however, is only a suggestion. I have not demonstrated it. All of the tissue elements in the diseased roots undergo hypertrophy, while some of them are subject to special changes in form as well as direction of growth.

"The parenchyma cells which normally have their tangential diameter greater than the radial are so changed that the radial diameter is the greater. This change in form of the parenchyma cells seems to obtain in nearly all of the parenchyma in the gall whether very near a cyst or distant from it. The increase in number of the wood and vascular cells of the central cylinder takes place though the cyst may not be located in or very near it. In such cases the fibres and ducts have their normal longitudinal direction. But if a cyst is located in or very near the central cylinder the ducts are turned in their direction of growth perpendicular to the axis of the root, bent around the cyst and then converge on the peripheral side, when, left, without any controlling influence over their direction of growth they often perform very curious evolutions through the parenchymatous tissue in all directions."

My results are not altogether in harmony with those given by Atkinson. Some of the nematodes stop in the cortex of the roots while others penetrate to the center of the axis cylinder which makes it necessary to consider the action of the nematodes on these two sets of tissues.

The writer agrees with Atkinson in the statement that the nematodes are found in all the tissues of the galls but as a result of studies on very young galls, the writer is of the opinion that the galls originate in the very young roots and at a period when most of the cells have retained the power of division. The writer is very doubtful if the parasites ever penetrate into the fibrous and tracheary tissues. The galls appear to originate in the young cortical tissues. As a result of the irritation by the parasites the fibrous and tracheary

tissues in the axis cylinder become distorted and sometimes displaced; new fibrous and tracheary tissues are formed in the cortical regions.

The size and shape of the galls depends primarily on the number of parasites in the roots and to some extent on their position in the roots and the age of the roots at time of infection. The writer is of the opinion that roots of more than one millimeter are rarely if ever infested.

The writer disagrees with Atkinson in his statement that "all the tissue elements in the diseased roots undergo hypertrophy". There is no hypertrophy of the tracheary tissue but new tracheary tissue is formed from the cortex. The writer also disagrees with Atkinson's statement that "the parenchyma cells which normally have their tangential diameter greater than the radial are so changed that the radial diameter is the greater". This statement is sometimes true especially in the very large galls which contain a large number of the parasites.

There is an enlargement of the roots due primarily to the excessive division and growth of the cells of the cortex. In most cases these cells are only slightly modified. Those next to the nematodes are somewhat smaller than normal. The larger cells may be slightly shorter (i. e. isodiametric) than in normal roots.

The cells in the axis cylinder do not increase in number but are pushed out of place by the growing nematodes.

There is a tendency for an increase in the amount of tracheary cells at the expense of the fibrous cells. Sometimes the axis cylinder is supplanted by the nematodes.

One part of an axis cylinder may be distorted and the other part normal.

The final result of the stimulation by the parasite is great masses of cells, the formation of new fibrous and tracheary tissues which are intermingled in the parenchyma in an irregular manner.

Atkinson compared the nematode galls with those caused by *Plasmodiophora brassicae* as follows:

"But if we take a thin transverse section of an enlarged root of each and compare them all resemblance vanishes. In a cross section of 'club-foot' the first thing to attract attention is the great number of yellowish plasmodia, or else the spore masses within large cells, distributed all through the tissues. If the section is from an enlargement of a lateral root, unless very large, there will be little else to attract the attention when compared with a healthy root unless it be a slight enlargement of some of the other cells. The general char-

acter of the root structure is but little changed. The tracheral tissue of the axis cylinder, but little attacked is arranged in the same stellate form which we find it in a healthy root. The ducts, even when immediately in contact with cells containing plasmodia, are not turned from their longitudinal direction, or if so only slightly. The cells are not elongated and curved around the enlarged cells containing the plasmodium, but resemble the normal arrangement of small cells around a large one. Nor is the radial diameter of the parenchymatous cells proportionately increased, but if the cells are enlarged it is usually a proportionate or nearly symetrical enlargement.''

The above comparison is very satisfactory for a comparison of sections of galls well advanced in their development. However, comparisons of the old galls are of very little value. Comparisons of young galls would no doubt give us some information concerning the developmental history. The writer has not had an opportunity of studying the *Plasmodiophora brassicae* galls but judging from the description of the histology by Lutman (**2**) and Kunkel, (**4**) they are much more simple in structure than the galls caused by nematodes. Lutman said:

''The difference between the cross-sections from a healthy root and those from a diseased one lies in the presence in the greatly hypertrophied cells of the cortical parenchyma of an opaque, colorless, finely granular plasma. The cortex is greatly increased in size and this enlargement is due to an increase in the number of the cells as well as to hypertrophy of the individuals. The vessels are often bent and shoved out of their proper position in diseased roots, but, as in normal ones, only contain air. The hypertrophied cells become filled in the older roots with small round spores of the organism to which Woronin gave the name of *Plasmodiophora Brassicae.*

Kunkel does not give us the history of the development of the very young galls caused by *P. brassicae,* but he said:

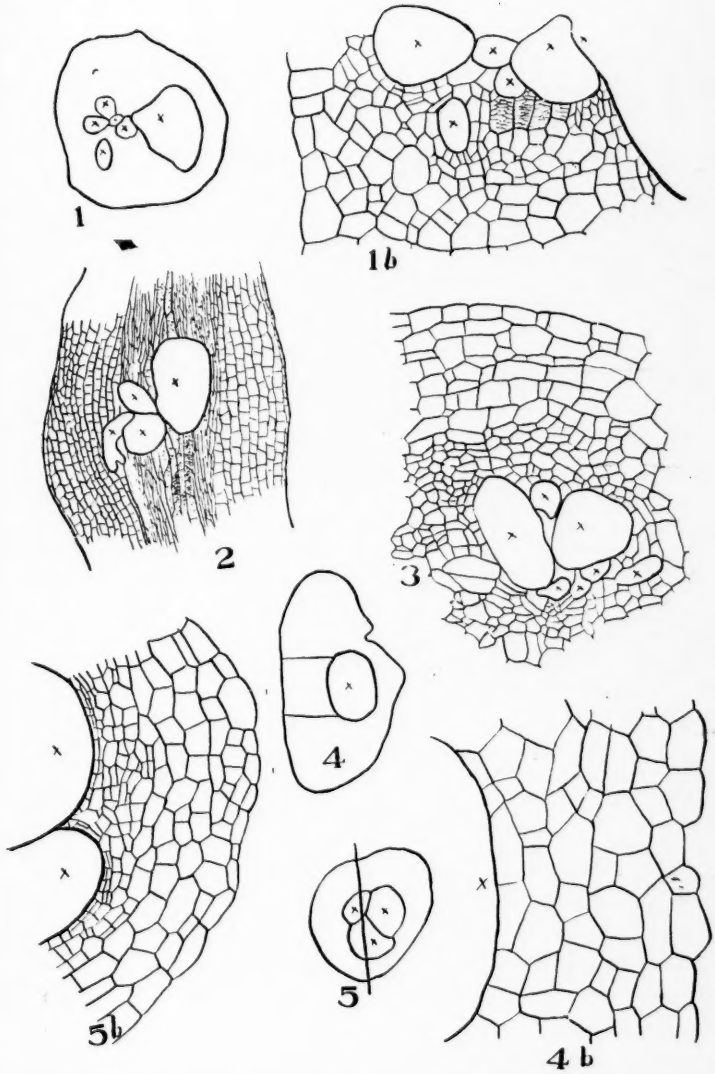
''Many of the host cells are very much enlarged, especially near the point of original infection. During the sixteenth and seventeenth days the parasite spreads still farther into the healthy tissues of the stem. It has not penetrated very much deeper, however, and does not seem to be able to attack the woody parts, at least to any very considerably extent.

''While the thickening of the cortex is accomplished more by cell growth than by cell multiplication, the swelling in the cambium region is brought about largely by an increase in the number of cells.''

He also stated that the cambium contributed to the enlargement, that the cortex grew, that the cortical cells were enlarged while those of the cambial region were small and that the parasite penetrated the rays and stimulated the cells so that the bundles were forced apart.

It appears to the writer that the galls resulting from nematodes are much more complex than the galls caused by *P. brassicae.*

PLATE XXIII



Summary

1. Root galls caused by nematodes (*Heterodera marioni*) originate as a result of attacks on meristematic tissues of very young roots.

2. The parasite may locate in the cambium or cortex or penetrate in the medullary rays.

3. There is no evidence that the parasite can attack fully formed fibrous and tracheary tissues or that it can stimulate their growth, but it can push them out of place.

4. The cambium, cortex and medullary rays are stimulated to excessive cell division and tracheary tissue is formed in the cortex.

5. The cells next to the parasites during the period of gall formation are very small as a result of excessive cell division while the more remote cells are much larger.

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Explanation of Plate

Fig. 1. Cross section of small root showing location of nematodes.

Fig. 1 *b*. Part of same showing the effect on the cells.

Fig. 2. Longitudinal section of small root showing location of nematodes.

Fig. 3. Cross section of a root showing location of nematodes. Note there is no tracheary tissue.

Fig. 4. Cross section of a root. Note there is no tracheary tissue.

Fig. 4 *b*. Part of same. Note the enlarged parenchyma cells.

Fig. 5. Cross section of small root. Note there is no tracheary tissue.

Fig. 5 *b*. Part of same. Note the parenchyma cells.